

program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

(a) modelling the parent alpha-amylase on an X-ray crystallographic three-dimensional structure of an alpha-amylase having the sequence of SEQ ID Nos: 2, 4, 6, or 13, or having a sequence at least [70 %] 80% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program using default values for GAP penalties, to produce a modelled three-dimensional structure of the parent alpha-amylase;

(b) identifying in the modelled three-dimensional structure obtained in step(a) at least one structural part of the parent wherein an alteration in said structural part is predicted to result in [said] an altered property, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca²⁺-dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(d) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase, wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.

72. (Amended) A method of constructing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13 or has a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

(a) modelling the parent alpha-amylase on an X-ray crystallographic three-dimensional structure of an alpha-amylase having the sequence of SEQ ID Nos: 2, 4, 6, or 13, or having a sequence at least [70%] 80% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program using default values for

GAP penalties, to produce a modelled three-dimensional structure of the parent alpha-amylase;

(b) comparing the modelled three-dimensional structure obtained in step (a) with a three-dimensional structure of an unrelated alpha-amylase, wherein the unrelated alpha-amylase differs from the parent alpha-amylase in [said] at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+} -dependency and specific activity;

(c) identifying a structural part of the modelled three-dimensional structure obtained in step (a) which is different from the three-dimensional structure of the unrelated alpha-amylase and which is predicted to be relevant to said property,

(d) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(e) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase, wherein the variant has alpha-amylase activity and has one or more altered properties as compared to the parent alpha-amylase.

Add new claims 73-75 reading as follows:

--73. A method of producing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13, or has a sequence at least 70% homologous to said sequence when homology is determined by the GAP program (Genetic Computer Group, Version 7.3) using default values for GAP penalties, said method comprising

(a) modelling the parent alpha-amylase on a first modelled three-dimensional structure to produce a second modelled three-dimensional structure, wherein the first-modelled structure is obtained by modelling an alpha-amylase on an X-ray crystallographic three-dimensional structure of an alpha-amylase having the sequence of SEQ ID Nos: 2, 4, 6, or 13, or having a sequence at least 80% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program using default values for GAP penalties;

(b) identifying in the second modelled three-dimensional structure obtained in step (a)

at least one structural part of the parent wherein an alteration in said structural part is predicted to result in an altered property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+} -dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(d) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase,

wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.—

--74. A method of constructing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID Nos: 2, 4, 6, or 13 or has a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, said method comprising

(a) modelling the parent alpha-amylase on a first modelled three-dimensional structure to produce a second modelled three-dimensional structure, wherein the first-modelled structure is obtained by modelling an alpha-amylase on an X-ray crystallographic three-dimensional structure of an alpha-amylase having the sequence of SEQ ID Nos: 2, 4, 6, or 13, or having a sequence at least 80% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program using default values for GAP penalties;

(b) comparing the second modelled three-dimensional structure obtained in step (a) with a three-dimensional structure of an unrelated alpha-amylase, wherein the unrelated alpha-amylase differs from the parent alpha-amylase in at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+} -dependency and specific activity;

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(c) identifying a structural part of the second modelled three-dimensional structure obtained in step (a) which is different from the three-dimensional structure of the unrelated alpha-amylase and which is predicted to be relevant to said property,

(d) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(e) expressing the modified nucleic acid to produce the variant alpha-amylase, wherein the variant has alpha-amylase activity and has one or more altered properties as compared to the parent alpha-amylase.

--75. A method of constructing a variant alpha-amylase having an altered property relative to its parent, said method comprising

(a) providing the sequences of a first and second alpha-amylase, each having a sequence selected from the group consisting of the sequence of SEQ ID Nos: 2, 4, 6, or 13 and a sequence at least 70% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program (Genetic Computer Group, Version 7.0) using default values for GAP penalties, wherein said first and second alpha-amylases differ in at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+} -dependency and specific activity;

(b) individually modelling said first and second alpha-amylases on an X-ray crystallographic three-dimensional structure of an alpha-amylase having the sequence of SEQ ID Nos: 2, 4, 6, or 13, or having a sequence at least 80% homologous to the sequence of SEQ ID Nos: 2, 4, 6, or 13 when homology is determined by the GAP program using default values for GAP penalties, to produce a first and second modelled three-dimensional structure, respectively;

(c) comparing said first and second modelled three-dimensional structures to identify a structural part of the first modelled three-dimensional structure which is different from the corresponding part of the second modelled three-dimensional and which is predicted to be relevant to said property,

(d) modifying the sequence of a nucleic acid encoding the first alpha-

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